PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
Case 21246		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/03862	14.04.2003	22.04.2002

International Patent Classification (IPC) or both national classification and IPC
C12N9/02

Applicant
DSM IP ASSETS B.V. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12.11.2003	28.07.2004

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/03862

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-18 as originally filed

Claims, Numbers

1-13 received on 22.04.2004 with letter of 19.04.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations

see separate sheet

1. The present application discloses an aldeyde dehydrogenase which is characterized by its phyico-chemical properties. The enzyme was isolated from a microorganism belonging to the genus Gluconobacter (DSM 4025).

2. Saito et al. (Biotechnology and Bioengineering, vol. 58, April/May 1998, p. 309-315; D1) disclose a sorbosone dehydrogenase (an aldehyde dehydrogenase) having a molecular weight of 55 kDa (p. 311, right col., first paragraph). No further physico-chemical characteristics are disclosed.
However, the enzyme of D1 does not appear to accept D-glucusone or D-glucose as a substrate (Hoshino et al., referred to in D1 on p. 311, right col., end of first paragraph).

None of the availabe documents suggests the existence of an enzyme as characterised in claim 1.

The aldehyde dehydrogenase of the present application therefore appears to be novel and based on an inventive activity.

1. (Amended) A purified aldehyde dehydrogenase having the following physico-chemical properties:

a) Molecular weight of 100,000 ± 10,000 Da (consisting of two homologous subunits) or molecular weight of 150,000 ± 15,000 Da (consisting of three homologous subunits), where each subunit has a molecular weight of 55,000 ± 2,000 Da);

b) Substrate specificity: active on L-sorbosone, D-glucosone, D-glucose, D-xylose;

c) Cofactor: pyrroloquinoline quinone (PQQ),

d) Optimum pH of from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbosone) or optimum pH of about 9.0 (for the production of 2-keto-L-gulonic acid from L-sorbosone),

e) Inhibitors: Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}, and monoiodoacetate.

2. The aldehyde dehydrogenase according to claim 1, which is derived from a microorganism belonging to the genus *Gluconobacter* which is capable of producing said aldehyde dehydrogenase.

3. The aldehyde dehydrogenase according to claim 2, wherein the microorganism is *Gluconobacter oxydans* having the identifying characteristics of the strain *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

4. The aldehyde dehydrogenase according to claim 3, wherein the microorganism is *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

5. A process for producing an aldehyde dehydrogenase having the following physico-chemical properties:

a) Molecular weight of 100,000 ± 10,000 Da (consisting of two homologous subunits) or molecular weight of 150,000 ± 15,000 Da (consisting of three homologous subunits), where each subunit has a molecular weight of 55,000 ± 2,000 Da);

b) Substrate specificity: active on aldehyde compounds,

c) Cofactor: pyrroloquinoline quinone (PQQ),

d) Optimum pH of from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbosone) or optimum pH of about 9.0 (for the production of 2-keto-L-gulonic acid from L-sorbosone),

e) Inhibitors: Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}, and monoiodoacetate,

which comprises cultivating a microorganism belonging to the genus *Gluconobacter*, which is capable of producing the aldehyde dehydrogenase having the above properties, in an aqueous nutrient medium under aerobic conditions, disrupting the cells of the microorganism, and

isolating and purifying the aldehyde dehydrogenase from the cell-free extract of the disrupted cells of the microorganism.

6. The process according to claim 5, wherein the reaction is carried out at a pH of from about 5.5 to 9.0 and at a temperature of from about 20 to about 50°C.

7. A process for producing a carboxylic acid and/or its lactone from its corresponding aldose which comprises contacting the aldehyde with the purified aldehyde dehydrogenase having the following physico-chemical properties:

a) Molecular weight of 100,000 ± 10,000 Da (consisting of two homologous subunits) or molecular weight of 150,000 ± 15,000 Da (consisting of three homologous subunits), where each subunit has a molecular weight of 55,000 ± 2,000 Da);

b) Substrate specificity: active on aldehyde compounds,

c) Cofactor: pyrroloquinoline quinone (PQQ),

d) Optimum pH of from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbosone) or optimum pH of about 9.0 (for the production of 2-keto-L-gulonic acid from L-sorbosone),

e) Inhibitors: Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}, and monoiodoacetate,

or cell-free extract prepared from a microorganism belonging to the genus *Gluconobacter* which is capable of producing the aldehyde dehydrogenase having the above properties in the presence of an electron acceptor.

8. The process according to claims 5 to 7, wherein the microorganism is *Gluconobacter oxydans* having the identifying characteristics of the strain *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

9. The process according to claim 8, wherein the microorganism is *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

10. The process of claim 7, wherein the lactone is vitamin C, the carboxylic acid is 2-keto-L-gulonic acid and the aldose is L-sorbosone.

11. The process according to any one of claims 7 to 10, wherein the reaction is carried out at a pH of from about 5.5 to about 9.0 and at a temperature of from about 20 to about 50°C for the production of vitamin C and 2-keto-L-gulonic acid, respectively.

12. The process according to any one of claims 7 to 11, wherein the reaction is carried out at a pH of from about 6.5 to about 8.0 and a temperature of from about 20 to about 40°C for the

production of vitamin C, and at a pH of about 9.0 and a temperature of from about 20 to about 30°C for the production of 2-keto-L-gulonic acid.

13. The use of the purified aldehyde dehydrogenase of claim 1 in the process for the production of a carboxylic acid and/or its lactone from its corresponding aldose which comprises contacting the aldehyde with said purified aldehyde dehydrogenase or cell-free extract prepared from a microorganism belonging to the genus *Gluconobacter* which is capable of producing said aldehyde dehydrogenase in the presence of an electron acceptor.